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Application of novel AIE materials in fluorescence probes

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Abstract. Fluorescence bioimaging is considered an indispensable technique in biological research due to its high sensitivity, excellent spatial and temporal resolution, non-invasiveness, rapid and real-time responsiveness, as well as its ease of accessibility. Among these, fluorescence probes play a pivotal role in fluorescence imaging technology thanks to their strong specificity, high sensitivity, rapid response, and straightforward implementation. Aggregation-Induced Emission (AIE) molecules exhibit a unique phenomenon: they emit weak or no fluorescence when dissolved in a solution but become highly luminescent under aggregated/clustering conditions. Leveraging this, fluorescent probes with AIE characteristics often demonstrate fluorescence activation under conditions of spontaneous aggregation or binding with analytes, showcasing high sensitivity and exceptional signal-to-noise ratios. Consequently, fluorescence probes based on the distinctive optical properties of AIE hold immense potential for applications in fluorescence bioimaging. This paper primarily investigates the synthesis properties and applications in biosensing of a series of novel organic fluorescence probes with aggregation-induced emission (AIE) characteristics. By synthesizing a range of compounds with AIE properties and experimentally determining their photophysical properties and molecular structures, the study explores the potential applications of these compounds in DNA recognition, protein detection, small molecule recognition, cellular pH detection, and bioimaging.

Keywords: organic fluorescence probes, aggregation-induced emission, biosensing, DNA recognition, protein detection, bioimaging.

1. Introduction

Fluorescence bioanalysis is an important technique to study the interaction between organisms and to analyze the biological structure and interaction process by using fluorescent probes [1]. Organic fluorescence probes are important signal tools for fluorescence sensing and optical imaging techniques. Generally, fluorescence probes used for biological analysis require high selectivity and sensitivity.

The light emission of conventional organic fluorescent probes is prone to quenching, that is, aggregation-caused quenching (ACQ phenomenon) [2]. In order to avoid the generation of ACQ effect, Tang of Hong Kong University of Science and Technology synthesized a series of fluorescent molecules in 2001. The performance of these molecules is completely opposite to the ACQ effect. Such molecules basically do not emit fluorescence when they are in solution but emit strong fluorescence when they are in aggregation or solid state. He named this phenomenon Aggregation-induced Emission (or AIE) [3].

In recent years, more and more studies have been conducted based on AIE molecules as new functional organic fluorescence probes for bioanalysis. In the work, the following will introduce the

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organic fluorescence molecules with AIE properties and the application of AIE organic molecules in the field of fluorescence bioanalysis

2. DNA Recognition

2.1. Design of DNA Fluorescence Probes

AIE Fluorescence Probes can be used for detecting and identifying DNA sequences, and their design and synthesis are crucial steps in constructing efficient DNA sensors and bioimaging probes. Novel AIE fluorescence probes can effectively recognize G-quadruplexes formed by single-stranded DNA rich in guanine. Figure 1 shows a series of positively charged TPE derivatives Biology to identify the G4 structure. Due to electrostatic interactions, the probes can be attracted to aggregate around single-stranded DNA, emitting strong fluorescence.

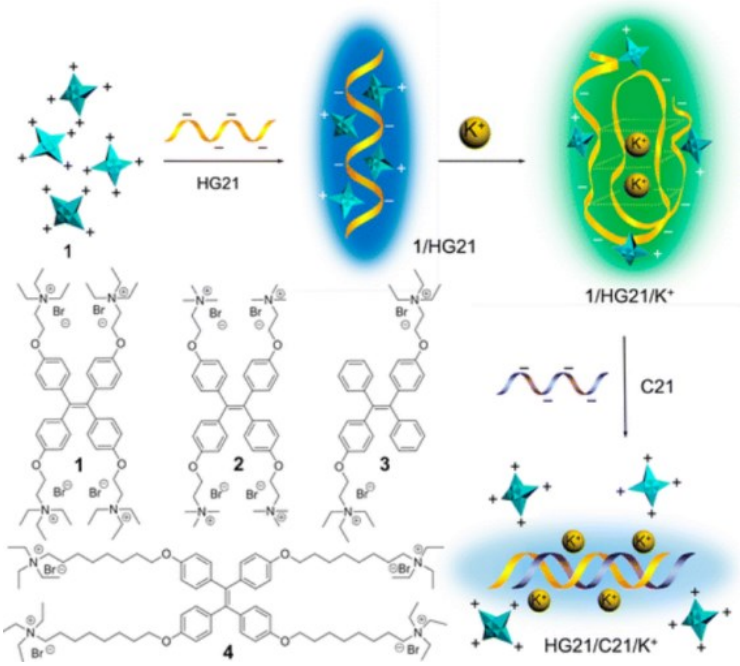

Figure 1. AIE Probe Structure and its Application of Probe 1 in DNA Analysis [4].

In the synthesis of DNA probes, commonly used methods include chemical synthesis and enzymatic synthesis [5]. Chemical synthesis involves synthesizing the probe sequence through chemical reactions, with solid-phase synthesis being the most frequently used method. Solid-phase synthesis entails adding nucleotide units step by step onto a synthesis scaffold to form the target sequence through chemical reactions. Enzymatic synthesis involves using DNA polymerases to synthesize the target sequence, with polymerase chain reaction (PCR) being the most commonly used method. PCR allows rapid amplification of the target sequence and introduces labels or modifications during amplification, facilitating probe synthesis.

The advantages of AIE probes lie in their high sensitivity, and graphene oxide(GO) can effectively enhance probe selectivity, enabling AIE probes to specifically identify DNA. Figure 2 shows that GO can quench AIE probes well and Calf thymus DNA(ct-DNA), as a naturally occurring double-stranded DNA, can competitively absorb probes from GO, thus forming a ct-DNA and probe aggregation complex. The design and synthesis of DNA probes are pivotal in biosensing research. Through rational design and selection of suitable synthesis methods, DNA probes with high sensitivity and selectivity can be obtained, providing robust support for DNA recognition and biosensing research.

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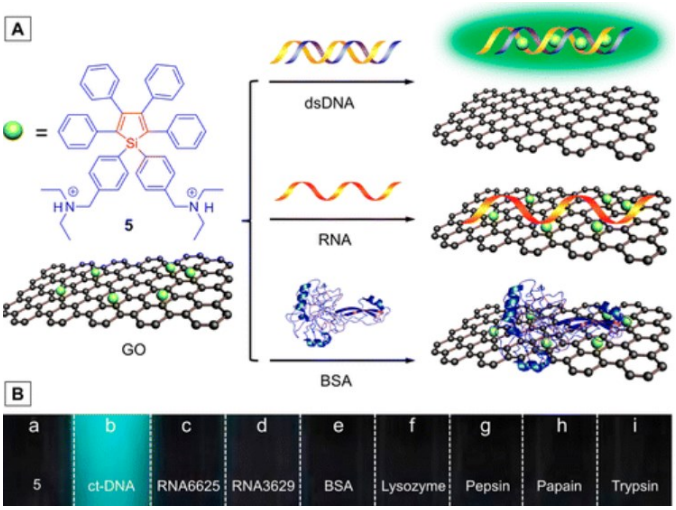

Figure 2. (A) Schematic representation of the selective recognition of Y-shaped DNA by Probe 5 and GO. (B) Fluorescence image of the Probe 5-GO complex under UV light exposure [6].

2.2. Application Research of DNA Recognition

The application research of DNA recognition mainly includes the following aspects:

Firstly, novel AIE organic fluorescence probes for DNA analysis were utilized. These novel AIE organic fluorescence probes possess excellent fluorescence performance and selectivity, enabling the determination and analysis of DNA through their interactions with DNA [7]. The design and synthesis of such probes form the foundation of DNA recognition application research. By modulating the structure and properties of the probes, specific recognition and analysis of DNA can be achieved. The scientists used AIE probes to label deoxyribonucleotides by fluorescent nucleic acid synthesis to improve the labeling, thereby avoiding the ACQ effect of traditional probes.(see Figure 3)

Secondly, investigate the mechanism of DNA recognition using novel AIE organic fluorescence probes. By studying the interaction mechanism between novel AIE organic fluorescence probes and DNA, the interaction mode and characteristics between the probes and DNA can be unveiled, providing a theoretical basis for designing more efficient DNA probes. Additionally, studying the changes in probe fluorescence performance and its binding form with DNA can reveal the relationship between DNA structure and function.

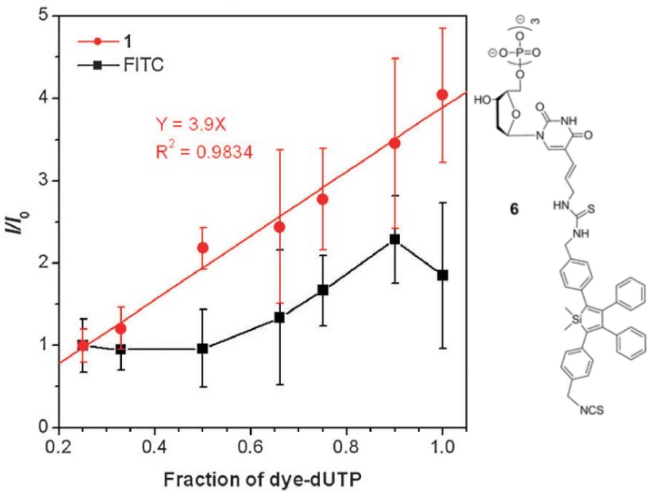

Figure 3. Fluorescence Normalized Spectrum of DNA with Probe-UTP [8].

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3. Protein Detection

3.1. Recognition Research of Protein Using AIE Probes

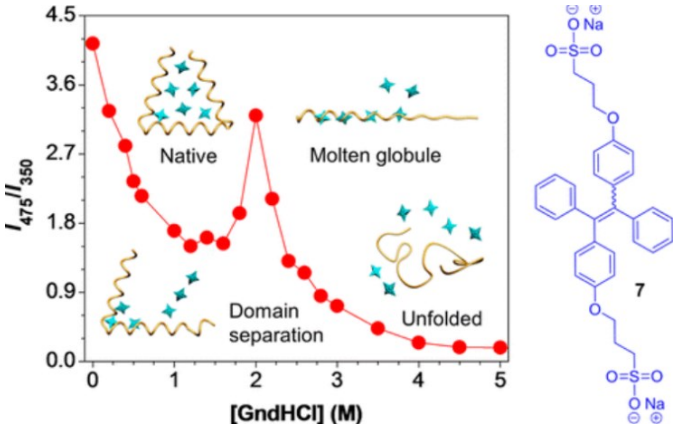

Figure 4. Fluorescence Relative Intensity of Protein HSA and Probe 7 at Different Concentrations of Guanidine Hydrochloride (GndHCl) [9].

Protein labeling and purification are commonly used methods in biological research, playing a significant role in studying the structure and function of proteins. Currently, there are various methods for protein labeling and purification that are widely applied in biological research.

AIE probes enhance fluorescence through electrostatic aggregation, enabling protein analysis. For instance, when a protein begins to unfold, energy transfers to the probe. By detecting the probe, changes in the protein's conformation can be observed. As shown in Figure 4, taking human serum albumin (HSA) as an example, probe 7 can monitor the conformational change of specific proteins. In the phosphoric acid buffer, the fluorescence energy of probe 7 increased with the addition of HSA. AIE materials' selective recognition properties towards proteins can be utilized to synthesize fluorescence probes like BATPS. BATPS is water-soluble and non-fluorescent in water; however, it exhibits significant fluorescence when extensively aggregated. This property of BATPS allows precise cell membrane imaging (see Figure 6). Through research, this novel fluorescence probe addresses the cumbersome and imprecise aspects of traditional probes, offering a more effective tool for disease detection.

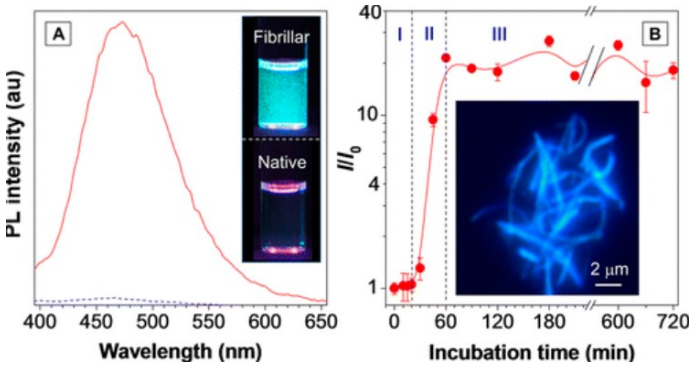

Figure 5. (A) Photoluminescence images of Probe 7 on native (blue dashed line) and fibrillar (red solid line) bovine insulin. (B) Fluorescence changes during the fibrillation process of bovine insulin [10].

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3.2. Application Research of Protein Detection

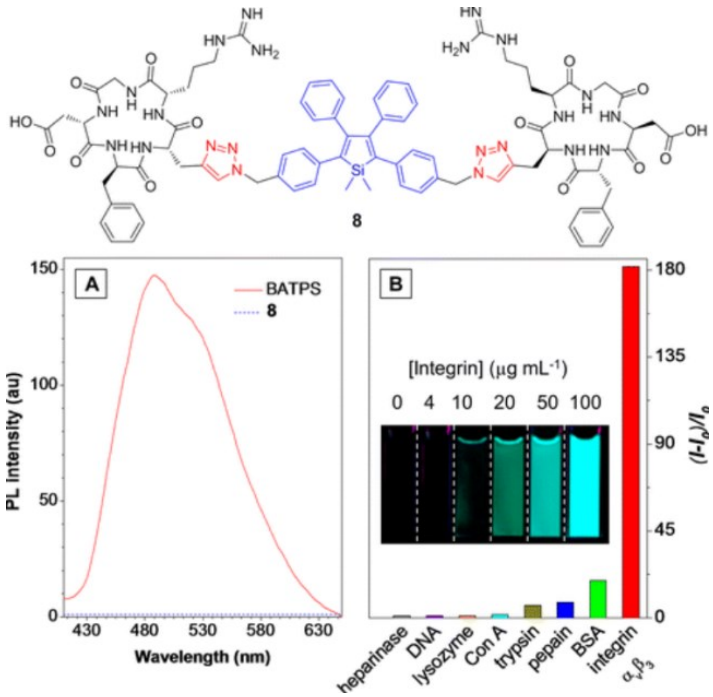

Figure 6. (A) Photoluminescence spectra of BATPS and its Probe 8 in DMSO and water. (B) Fluorescence response of Probe 8 to different proteins and their DNA. I and I0 represent the fluorescence values of Probe 8 in 100 and 0 μg/mL analyte, respectively. The inset shows fluorescence images of Probe 8 at different concentrations with αvβ3 [11].

Proteins are essential molecules within living organisms, playing critical roles in cell structure and function. Therefore, accurately and rapidly detecting the presence and quantity of proteins holds significant importance in biological research and clinical diagnostics. Over the past few decades, researchers have developed various methods to detect proteins, including immunological methods, mass spectrometry, and electrochemical methods. However, these traditional detection methods often face limitations in terms of specificity, sensitivity, and real-time capabilities. As shown in Figure 5A, the phosphoric acid buffer solution of probe 7 binds to fibrous bovine insulin and emits intense fluorescence and Figure 5B shows that the shaped protein shape can be well recognized by probe 7.

In recent years, with the rapid advancement of fluorescent probe technology, fluorescence-based protein detection methods have become a growing research focus. Novel AIE organic fluorescence probes, as functional compounds with excellent fluorescence performance, have been widely applied in the field of protein detection.

Firstly, researchers have designed highly sensitive protein detection methods through the synthesis of novel AIE organic fluorescence probes. These probes exhibit significant fluorescence enhancement or quenching upon binding to specific proteins, enabling protein detection and quantitative analysis. Compared to traditional methods, this AIE organic fluorescence probe-based detection approach offers higher sensitivity and specificity.

Secondly, researchers have explored the application of AIE organic fluorescence probes in protein structure recognition. By introducing specific structural units or functional groups into probe molecules, selective and specific recognition of different proteins can be achieved. This provides essential tools and means for further studying protein structure and function. As shown in Figures 7B and C, this probe can monitor the phenomenon of cell apoptosis induced by staphylosporine, and this probe can also monitor apoptosis in real-time by monitoring casPASE 3.

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Furthermore, AIE organic fluorescence probes have also found extensive application in bioimaging studies for protein detection. By tagging probe molecules onto specific proteins, real-time imaging of protein expression, localization, and function can be achieved at the cellular and tissue levels. This fluorescence imaging technique offers crucial insights into various aspects of protein research.

In conclusion, protein detection methods based on AIE organic fluorescence probes exhibit clear advantages in terms of sensitivity, specificity, and real-time capabilities. With further research and application of AIE organic fluorescence probes, it is believed that this innovative protein detection method will play a significant role in biological research and medical diagnostics.

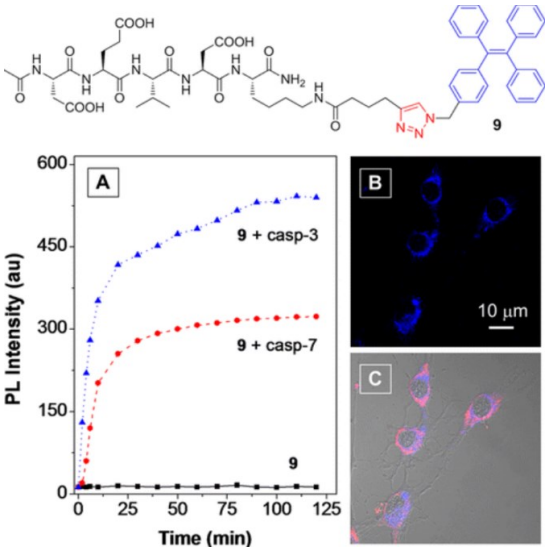

Figure 7. (A) Response of Probe 9 to cysteine protease at different time points. (B) Fluorescence imaging of Probe 9 in camptothecin-induced MCF-7 cell apoptosis, and (C) Overlay image using a commercial stain (anti-caspase-3) [12].

4. Small Molecule Recognition

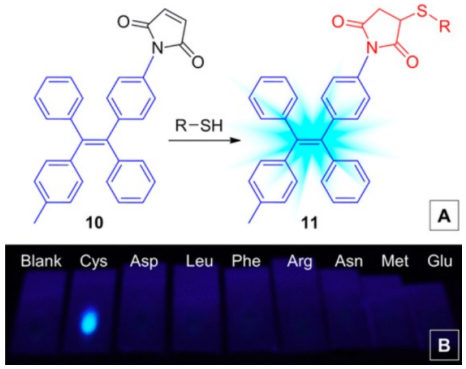

Figure 8. (A) Schematic representation of thiol compound monitoring. (B) Photo of thiol fluorescence recognition based on TLC [13].

4.1. Recognition of Small Molecules Using Novel AIE Fluorescent Probes

The design and synthesis of small molecule probes aim to selectively interact with target small molecules and generate specific signal responses by altering molecular structure and functional groups. The first step in designing a probe is to determine the structure and properties of the target small molecule, in order to select appropriate ligands and signal transduction units. Subsequently, based on the specificity of the target molecule, suitable fluorescent groups and fluorescence enhancers are chosen. Finally, through synthesis and purification methods, all components are combined to create a probe with

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target recognition and signal response capabilities. As shown in Figure 8, a maleamide labeled TPE (probe 10) was synthesized to recognize mercaptan.

In the design and synthesis of small molecule probes, some key issues need to be considered, such as selectivity and sensitivity of the probe. Selectivity refers to whether the probe can exclusively recognize the target small molecule with specificity, without interference from other substances. Sensitivity refers to the detection limit and response speed of the probe towards the target small molecule. To enhance selectivity and sensitivity, researchers adjust the structure and functional groups of the probe to improve its performance. Additionally, biomolecules such as peptides and nucleic acids can be used as recognition elements for the probe, imparting higher selectivity and sensitivity. As shown in Figure 9, TPE reacts with the addition of boric acid to form probe 12, and intensity increases when 0.1 mM D-glucose (Glu) is added.

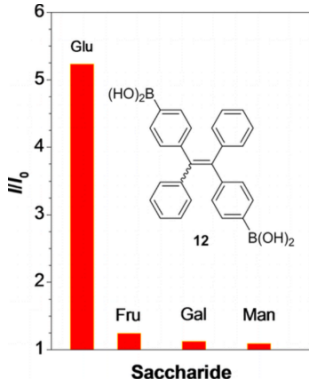

Figure 9. Fluorescence response of Probe 12 to different sugars. I0 represents the fluorescence intensity of Probe 12 at pH 12 [14].

5. Biological Imaging

The fundamental principle of biological imaging involves using specific probes to convert fluorescence signals into visible light signals through excitation and emission processes. The selection of probes plays a crucial role in imaging choices. In research, novel AIE (Aggregation-Induced Emission) organic fluorescence probes exhibit high luminescence efficiency and strong fluorescence stability. These probes are widely applied in the field of biological imaging due to their distinctive structure and luminescence mechanisms. Figure 10 shows Tang coated probe 13 with AIE performance with silica nanoparticles, which showed good bleaching resistance and very uniform particle size. As shown in Figure 11, Tang then designed probe 14-droped Fe3O4 nanocrystalline silica fluoro-magnetic nanoparticles with high fluorescence intensity and good biocompatibility, which can be applied to cell fluorescence imaging research and nuclear magnetic resonance imaging applications.

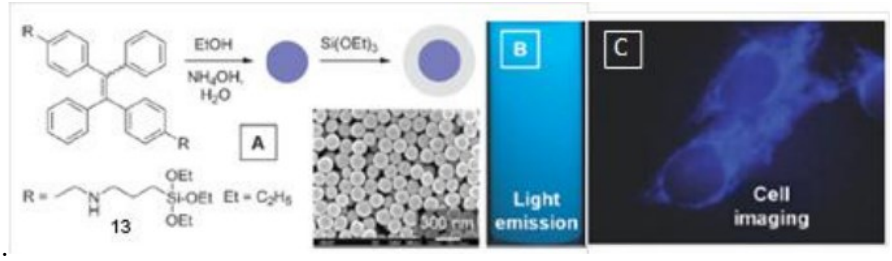

Figure 10. (A) Synthesis diagram of Probe 13 nanoparticles; (B) Image in solution under ultraviolet light illumination; (C) Imaging of Hela cells labeled with Compound 58 [15].

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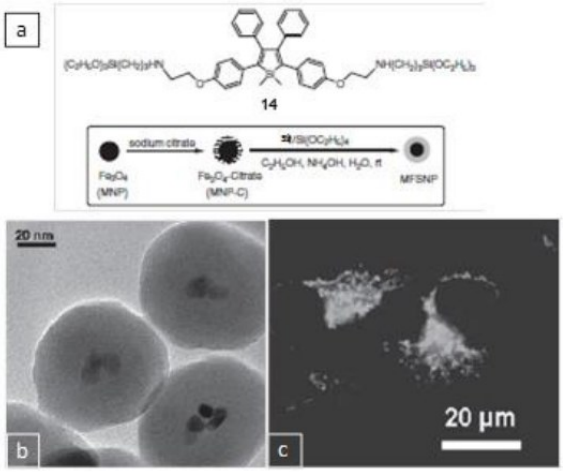

Figure 11. (a) Synthesis route of Probe 14 nanoparticles; (b) TEM image of Probe 14 nanoparticles; (c) Imaging of Hela cells using Probe 14 nanoparticles.[16]

Several theoretical models have been proposed regarding the luminescence mechanism of AIE organic fluorescence probes. Among them, the most widely used are the Restricted Intramolecular Rotation (RIR) model and the Aggregation-Induced Enhanced Emission (AIEE) theory. The Restricted Intramolecular Rotation model suggests that AIE organic fluorescence probes undergo self-aggregation in solution, and the limited rotational ability of aggregated organic molecules, due to poor redox capability, leads to enhanced luminescence. On the other hand, the Aggregation-Induced Enhanced Emission theory proposes that during the aggregation process of AIE organic fluorescence probes, hydrogen bonding interactions between molecules hinder non-radiative energy transfer, thereby increasing the fluorescence yield of organic molecules. The development of these theoretical models provides a foundation for understanding the luminescence mechanism of AIE organic fluorescence probes in biological imaging.

6. Conclusion

Novel AIE materials have effectively expanded the application scope and limitations of existing fluorescence probes. They offer a cost-effective, highly selective, and stable approach for areas such as biosensing, disease monitoring, and bioimaging. The synthesis properties of novel AIE organic fluorescence probes and their applications in biosensing research hold significant value in the field of cellular detection. These probes achieve high sensitivity and selectivity for intracellular detection by interacting with various biomolecules within cells. Through the application of these probes, researchers can gain a better understanding of cellular regulatory mechanisms and play a crucial role in disease diagnosis and treatment. In the future, with further development of AIE probe capabilities, their application is expected to become even more widespread in biological and medical research.

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